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Pathogenicity of Low and Highly Virulent *Chlamydia psittaci* Isolates for Specific-Pathogen-Free Chickens

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SUMMARY. In commercially raised poultry, chlamydiosis mostly seems to occur on turkey or duck farms, sometimes associated with zoonotic transmission and disease (psittacosis) in humans. However, *Chlamydia* infections are apparently emerging in chickens, and information on the virulence of *Chlamydia* in chickens is limited. Up-to-date *Chlamydia psittaci* genotypes B and D are most frequently found in broilers. We examined the pathogenicity of the well-characterized *C. psittaci* genotype B (CP3) and D (92/1293) strains in experimentally (aerosol) infected specific-pathogen-free chickens. Both strains caused conjunctivitis, rhinitis, and dyspnea. Pharyngeal and cloacal *C. psittaci* excretion was observed in all infected animals, indicative for systemic dissemination as proven by immunofluorescence staining of frozen tissue sections. Histopathologic lesions were present in all infected chickens. However, differences in pathology were observed. Genotype D led to mortality and more severe clinical signs and lesions as compared to genotype B, which showed lower virulence.

RESUMEN. Patogenicidad de aislamientos baja y alta virulencia de *Chlamydia psittaci* para aves libres de patógenos específicos. La clamidiosis puede presentarse en aves criadas comercialmente, principalmente en granjas de pavos y patos, a veces asociados con la transmisión zoonótica y la presentación de la enfermedad (psitacosis) en humanos. Sin embargo, las infecciones por *Chlamydia* aparentemente están surgiendo en los pollos y la información sobre la virulencia de *Chlamydia* en los pollos es limitada. Hasta la fecha, los genotipos B y D de *Chlamydia psittaci* son los más frecuentes en pollos de engorde. Se examinó la patogenicidad de las cepas bien caracterizadas de *C. psittaci* genotipo B (CP3) y genotipo D (92/1293) en aves libres de patógenos específicos infectados experimentalmente por aerosol. Ambas cepas causaron conjuntivitis, rinitis, y disnea. Se observó excreción faríngea y cloacal de *C. psittaci* en todos los animales infectados, indicativas de diseminación sistémica como fue demostrado por la tinción de inmunofluorescencia de cortes de tejido congelados. Las lesiones histopatológicas estaban presentes en todos los pollos infectados. Sin embargo, se observaron diferencias en la patología. El genotipo D ocasionó mortalidad además de signos clínicos y lesiones más graves en comparación con el genotipo B, que mostró una menor virulencia.

Key words: *Chlamydia psittaci*, chickens, poultry, broilers, pathology

Abbreviations: ATCC = American Type Culture Collection; BALT = bronchi-associated lymphoid tissue; dpi = day(s) postinfection; LPS = lipopolysaccharide; *ompA* = outer membrane protein A; PBS = phosphate buffered solution; SPF = specific pathogen free; $TCID_{50}$ = 50% tissue culture infective dose

Chlamydia psittaci is an obligate, intracellular gram-negative bacterium causing respiratory disease in poultry. *C. psittaci* is classified into the well-characterized outer membrane protein A (*ompA*) genotypes A–F and E/B. Genotypes B, C, D, F, and E/B have been found in chickens (6,8,19,25,26,27).

In commercially raised poultry, chlamydiosis mostly seems to occur on turkey or duck farms, sometimes associated with zoonotic transmission and disease (psittacosis) in humans. However, *Chlamydia* infections are apparently emerging in chickens. Dickx *et al.* (6) examined 10 randomly selected Belgian broiler breeder, broiler, and layer farms by a recombinant major outer membrane protein-based antibody ELISA (23) and found 98%, 95%, and 95% seropositive layers, broilers, and broiler breeders, respectively. Moreover, they demonstrated the presence of *C. psittaci* genotype D strains in the air of chicken hatching chambers and in Belgian and French broilers sampled at slaughter. Zoonotic transmission of genotype D strains to hatchery and abattoir employees did occur (6,7). In France, three cases of atypical pneumonia in individuals working at a French poultry slaughterhouse that processes guinea fowl, ducks, and especially chickens prompted Laroucau *et al.* (9) to conduct an epidemiological survey in 10 supplying poultry farms. Using a *Chlamydiaceae*-specific real-time PCR, chlamydial agents were detected in 12 of 18 (67%) investigated chicken flocks. Positivity for the flocks ranged between 10% and 100%. Rather unexpectedly, ArrayTube DNA microarray testing for further characterization of the chlamydial agents indicated the presence of an atypical chlamydial agent in seven chicken flocks, originating from six different breeders. Surprisingly, all chicken flocks appeared healthy. Recent data suggest that this new chlamydial agent could putatively be widespread in Australian, French, Greek, Croatian, Slovenian, and Chinese chicken flocks (12,28).

Information on the virulence of *Chlamydia* in chickens is limited. Up-to-date *C. psittaci* genotypes B and D were most frequently found in broilers (7,19,25). Beeckman *et al.* (5) already performed a study in chicken macrophages (HD11 cells) comparing host pathogen interactions of the low virulent genotype B reference strain CP3 (3,11) to the highly virulent genotype D strain (92/1293). CP3 was isolated in 1957 from a Californian pigeon (3), while 92/1293 was isolated in 1992 from Dutch diseased turkeys (18). The genotype D strain 1) clearly induced actin recruitment to the site of *Chlamydia* entry and invaded the host cells more efficiently, 2) initiated host cell degeneration at earlier time points, and 3) survived and proliferated better in macrophages when compared to the low virulent CP3 strain.

The purpose of the present research was to study the pathogenicity of CP3 and 92/1293 *in vivo* in specific-pathogen-free (SPF) chickens

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Table 1. Macroscopic lesion scoring system. Tissues with no lesions were scored 0.

Tissue	Lesion score 1	Lesion score 2	Lesion score 3
Conjunctiva	Congestion unilateral	Moderate congestion bilateral	Severe congestion bilateral
Conchae	Slightly congested	Severely congested	Severely congested + viscous mucus
Lung	Congestion bilateral	Congestion + gray foci unilateral	Congestion + gray foci bilateral
Thoracic air sac	Diffuse opacity	Focal fibrinous airsacculitis	Diffuse fibrinous airsacculitis
Abdominal air sac	Diffuse opacity	Focal fibrinous airsacculitis	Diffuse fibrinous airsacculitis
Pericardium	Serous pericarditis	Serous pericarditis	Serous adhesive pericarditis
Spleen	Slightly enlarged	Moderately enlarged	Severely enlarged + petechiae
Liver	Slightly congested	Moderately congested	Moderately congested + petechiae
Kidney	Slightly enlarged	Moderately enlarged	Severely enlarged
Intestine	Slightly congested	Moderately congested and fluid inside	Severely congested and fluid inside

and to compare the results with the previously obtained *in vitro* (HD11 cells) data of this pathogen (5), which according to recent literature seems to be emerging in chickens.

MATERIALS AND METHODS

C. psittaci. *C. psittaci* genotype D strain 92/1293 (18) and *C. psittaci* genotype B strain CP3 (ATCC VR-574) were used (3). *C. psittaci* was grown in Buffalo green monkey cells as previously described (20). Bacterial titration was performed by the method of Spearman and Kaerber to determine the 50% tissue culture infective dose ($TCID_{50}$) per ml (10).

Experimental infection. The experimental design was evaluated and approved by the Ethical Commission for Animal Experiments of Ghent University (EC 2010/054). Briefly, three groups of 22-day-old SPF chickens (Lohman, Cuxhaven, Germany) were individually tagged and housed in separate negative pressure isolators (IM1500, Montair, Sevenum, the Netherlands). At age 1 week, groups 1 and 2 were exposed for 1 hr to an aerosol containing 10^6 $TCID_{50}$ of *C. psittaci* genotype B (CP3) or genotype D (92/1293) suspended in PBS (5-μm droplets; Cirrus™ nebulizer; Lameris, Aartselaar, Belgium), respectively. A third group received an aerosol of PBS and served as noninfected control.

Clinical signs and macroscopic lesions. Clinical signs were daily recorded until 34 days postinfection (dpi). It was our purpose to euthanatize two birds per group at 2, 4, 6, 8, 10, 14, 17, 21, 24, 28, and 34 dpi for detailed examination. However, dead birds would be examined immediately regardless of the dpi. Macroscopic lesions were scored according to Table 1.

***C. psittaci* excretion.** Pharyngeal and cloacal excretion were determined by examining rayon-tipped, aluminium-shafted swabs (Colpan; Fiers, Kuurne, Belgium) provided with *C. psittaci* transport medium and used for sampling at euthanasia. Swabs were stored at −80 C until processed. *Chlamydia* excretion was monitored using standard procedures for culture and bacterial identification (IMAGEN™ immunofluorescence stain; Dakocytomation, Copenhagen, Denmark). The presence of *Chlamydia* was enumerated in five randomly selected microscopic fields (600×, Nikon Eclipse TE2000-E, Tokyo, Japan) and results were scored from 0 to 5. Score 0 indicated that no *C. psittaci* was present; score 1 was given when a mean of 1–5 elementary bodies was present; scores 2, 3, 4, and 5 were given when a mean of 1–5, 6–10, 11–20, and >20 inclusion positive cells was present.

***C. psittaci* replication in tissues.** At euthanasia, tissue samples of the conjunctiva, conchae, sinus, trachea, lungs, abdominal and thoracic air sacs, pericardium, spleen, liver, kidney, and jejunum were immersed in methocel (Methocel MC, Sigma, Bornem, Belgium), snap frozen in liquid nitrogen, and stored at −80°C until processed. Cryostat tissue sections (5 μm) were examined for the presence of *Chlamydia* by the IMAGEN immunofluorescence staining. The presence of *Chlamydia* was enumerated as for chlamydial excretion.

Immunohistochemistry and histopathology. At euthanasia, tissue samples of the conjunctiva, conchae, sinus, trachea, lungs, abdominal and thoracic air sacs, pericardium, spleen, liver, kidney, jejunum, and ovary/testis were fixed in formalin (4% buffered neutral formaldehyde), processed, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for histopathology. For immunohistochemistry, fixed samples were dehydrated after 24 hr and embedded in paraffin. Sections were stained with a *Chlamydiaceae* family-specific mouse monoclonal antibody directed against chlamydial lipopolysaccharide (LPS) (mLPS; clone ACI-P; Progen Biotechnik, Heidelberg, Germany). All slides were examined microscopically (Leitz, New York, NY). Histopathologic findings were graded (score 1: mild; score 2: moderate; or score 3: severe), while immunohistochemical findings were scored from 1 to 4, depending on the number of positive cells observed.

Statistics. Means of 2 birds per sample point (dpi) were pooled per infection group (CP3, 92/1293 or negative control) for the entire course of infection as monitored from 2 dpi until 34 dpi, and into one of three stages of infection: early infection (2, 4, and 6 dpi), midinfection (8, 10, 14, and 17 dpi), and late infection (21, 24, 28, and 34 dpi). Data were subject to the nonparametric Kruskal-Wallis one-way ANOVA test using SPSS® version 21 (IBM, Somers, NY). Significance was set at $P < 0.050$.

RESULTS

Clinical signs and macroscopic lesions. Non-infected controls (group 3) remained healthy throughout the experiment. All chickens in groups 1 and 2 showed respiratory disease. However, clinical signs were more severe in chickens infected with the highly virulent genotype D strain 92/1293 (group 2). Early infection with the genotype D strain was characterized by respiratory symptoms, which were exacerbated during midinfection and diminished but not completely resolved during late infection. Anorexia and mortality were only observed during midinfection. Respiratory symptoms were first observed at 3 dpi, when 4 of 20 remaining chickens showed conjunctivitis (scratched their eyes), and rhinitis (head shaking). By 8 dpi all chickens showed conjunctivitis, rhinitis, dyspnea, and watery droppings. Symptoms were most severe from 8 to 17 dpi. At that time all chickens showed anorexia and they were gasping and sitting down with drooping wings. Afterwards, only slight dyspnea (open beak breathing) and occasionally head shaking (rhinitis) could be observed until the end of the experiment at 34 dpi. Two chickens died (one at 8 dpi and the other at 9 dpi).

Watery droppings, anorexia, or mortality was not observed in genotype B–infected chickens. Respiratory symptoms due to a genotype B infection were less severe as compared to a genotype D infection, but also started with low incidence and severity during early infection, exacerbated during midinfection, and diminished during late infection. Clinical signs first appeared at 4 dpi, when 2 of 20 remaining animals showed conjunctivitis, rhinitis, and slight dyspnea. Conjunctivitis, rhinitis, and moderate dyspnea were observed in all chickens from 10 to 14 dpi. Afterwards, only

Table 2. Mean culture scores for pharyngeal and cloacal *C. psittaci* excretion and its presence in tissues in early, mid, and late infection stages of *C. psittaci*–infected chickens. Early infection: mean scores of two birds on 2, 4, and 6 dpi; midinfection: mean scores of two birds on 8, 10, 14, and 17 dpi; late infection: mean scores of two birds on 21, 24, 28 and 34 dpi.[A]

	Group 1 (CP3)				Group 2 (92/1293)			
Tissue	Early	Mid	Late	Overall	Early	Mid	Late	Overall
Pharynx	1.2	1.6	0.8	1.2a	2.2	2.5	1.0	1.9b
Cloaca	0.2	1.5a	0.6	0.8a	0.5x	2.4b,y	2.0xy	1.7b
Conjunctiva	0.3	0.5	0.1	0.3	0.7xy	1.3x	0.3y	0.7
Conchae	0.2	0.4a	0.1	0.2a	0.7	1.5b	0.9	1.0b
Sinus	1.0	0.4a	0.3	0.5	1.0	1.4b	0.5	1.0
Trachea	0.7	0.4a	0.3	0.4a	2.0	2.0b	0.8	1.5b
Lung	0.5	1.0	0.5a	0.7a	1.5	1.9	1.0b	1.5b
Thoracic air sac	0.7	1.0a	0.4a	0.7a	1.7	2.9b	1.9b	2.2b
Abdominal air sac	0.2x	1.3a,y	0.6xy	0.7a	1.5	2.8b	1.6	2.0b
Pericardium	0.3	0.4	0.1	0.3	0.5	1.3	0.9	0.9
Spleen	0.2	0.8a	0.8	0.6a	0.8	2.4b	1.3	1.5b
Liver	0.0	0.9	0.8	0.6	0.3	1.6	1.3	1.1
Kidney	0.5	0.6	0.0	0.4a	1.2	1.8	0.4	1.1b
Jejunum	0.0x	0.8y	0.5a,xy	0.5	0.0x	1.4y	1.4b,y	1.0

[A]Different lowercase letters (a or b) within a row indicate a significant difference between same stages of infection (early, mid, or late) at $P < 0.05$. Different lowercase letters (x or y) within a row indicate a significant difference between stages of infection (early, mid, or late) at $P < 0.05$.

moderate dyspnea was observed until 34 dpi. Mortality was not observed.

***C. psittaci* excretion.** Noninfected controls shed no *C. psittaci*. Pharyngeal and cloacal shedding started for both strains at 2 and 6 dpi, but was most pronounced in chickens infected with the highly virulent genotype D strain 92/1293 ($P < 0.05$). Besides this overall effect, cloacal excretion was specifically during midinfection significantly higher in genotype D than in genotype B–infected chickens. In addition, a genotype D infection resulted in a significantly higher cloacal excretion during midinfection as compared to early or late infection stages (Table 2).

***C. psittaci* replication in tissues.** *C. psittaci* was absent in tissues of the control group 3. Overall, infection with strain 92/1293 resulted in significantly higher chlamydial replication in the upper (conchae and trachea) and lower (lung and abdominal, and thoracic air sacs) respiratory tract, as well as in the spleen and kidney. Chlamydial replication during midinfection was also more pronounced ($P < 0.05$) in conchae, sinus, trachea, and abdominal and thoracic air sacs and spleen of strain 92/1293 compared to strain CP3-infected chickens. The same was observed in lung, thoracic air sac, and jejunum during late infection (Table 2). From 4 to 21 dpi, all euthanatized chickens of group 2 (strain 92/1293) contained *C. psittaci* throughout the upper and lower respiratory tract, whereas the upper and lower respiratory tract of animals of group 1 (strain CP3) was completely positive only on 8 and 10 dpi. Thus, 92/1293 replicated more intensively in the respiratory tract than CP3. Systemic dissemination of the infection was also more pronounced for group 2. For group 2, all examined tissues of chickens euthanatized on days 8, 10, 14, and 21 postinfection were positive. For group 1, day 10 postinfection was the only one whereupon all examined tissues were positive for both euthanatized chickens. Strain 92/1293 was thus more virulent than strain CP3, as 92/1293 replicated more intensively and during a longer period in several of the examined tissues.

Immunohistochemistry and histopathology. Group 1 and 3 were negative by immunohistochemistry. Thus, chlamydial LPS was found only in strain 92/1293–infected chickens, where it could be detected from 4 dpi until 32 dpi onwards. In this group, chlamydial LPS was demonstrated in all examined tissues, except for conjunctiva and jejunum. Its presence was most pronounced during midinfection and was highest in air sacs ($P > 0.05$) (Table 3). Although immunohistochemistry revealed systemic dissemination of strain 92/1293, scores and tissue tropism seemed less pronounced as demonstrated by the immunofluorescence stainings on cryosections. The highest scores were found on 10 dpi, which is in accordance with the results of the immunofluorescence staining on frozen tissue sections and of histopathologic lesions, which were also most severe on 10 dpi.

Hematoxylin-eosin staining revealed no lesions in tissues of the control group. Histopathologic changes were most pronounced in strain 92/1293-infected chickens, which also showed more severe clinical symptoms and chlamydial replication as compared to CP3-infected chickens. Fig. 1 illustrates pathological changes observed in the lungs of chickens infected with strain 92/1293 and strain CP3 during early and late infection. Overall, infection with strain 92/1293 resulted in more severe lesions in air sacs, pericardium, kidney,

Table 3. Mean score for the immunohistochemical detection of chlamydial lipopolysaccharide (LPS) in tissues of chickens infected with genotype D strain 92/1293 at early, mid, and late stage of infection. (Chlamydial LPS was not detected in tissues of chickens infected with genotype B strain CP3.) Early infection: mean scores of two birds on 2, 4, and 6 dpi; midinfection: mean scores of two birds on 8, 10, 14, and 17 dpi; late infection: mean scores of two birds on 21, 24, 28, and 34 dpi.

	Infection stage			
Tissue	Early	Mid	Late	Overall
Conjunctiva	0.0	0.0	0.0	0.0
Conchae	0.0	0.3	0.0	0.1
Sinus	0.0	0.3	0.0	0.1
Trachea	0.2	0.7	0.0	0.3
Lung	0.2	0.5	0.0	0.2
Thoracic air sac	0.5	2.2	0.5	1.1
Abdominal air sac	0.7	3.0	1.5	1.7
Pericardium	0.0	0.3	0.2	0.2
Spleen	0.0	0.7	0.2	0.3
Liver	0.0	0.7	0.0	0.2
Kidney	0.0	0.7	0.3	0.3
Jejunum	0.0	0.0	0.0	0.0
Ovary/testes	0.0	0.5	0.0	0.2

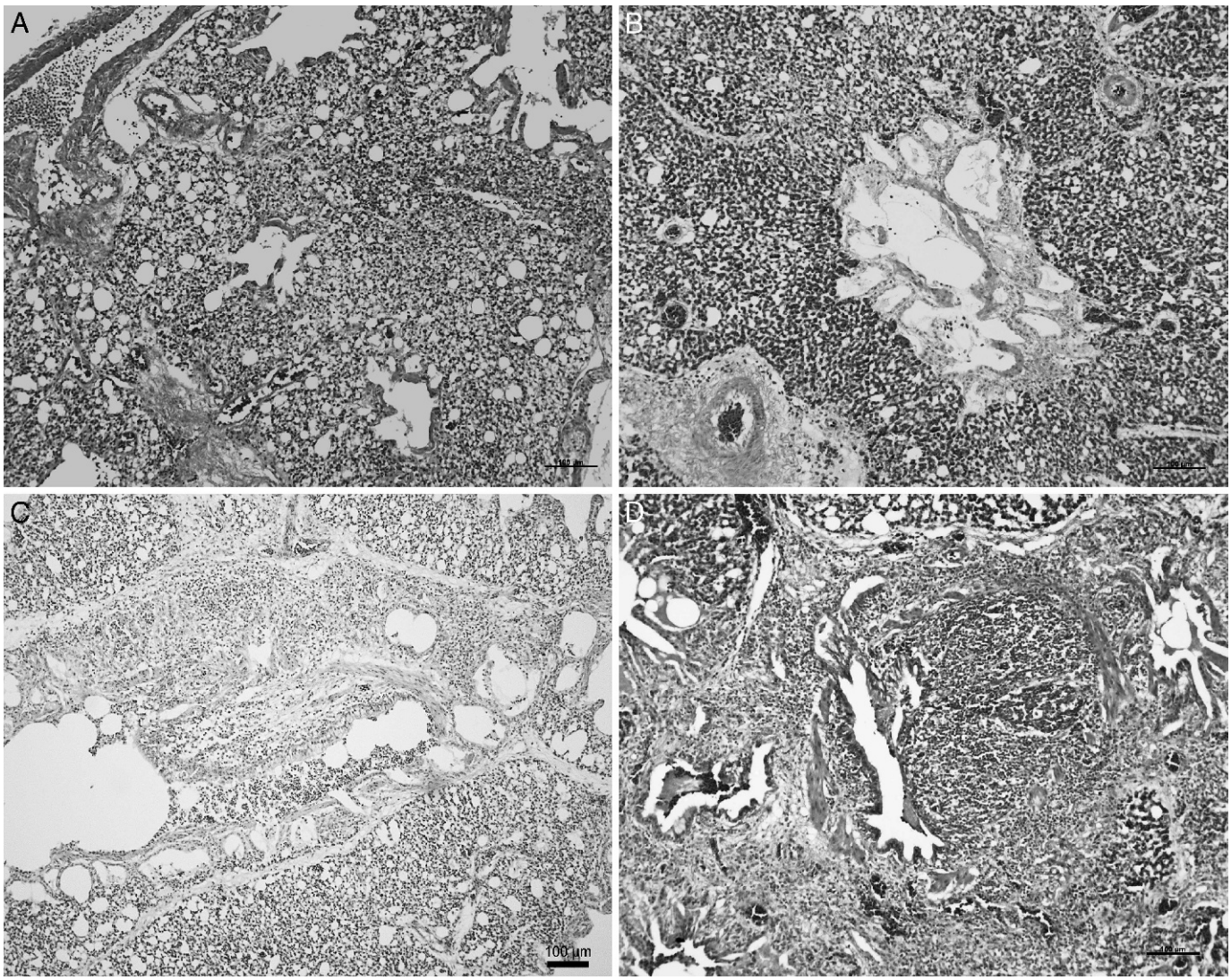

Fig. 1. Hematoxylin and eosin staining of chicken lungs infected with *C. psittaci* genotype D strain 92/1293 and genotype B CP3 at 6 and 21 dpi (10×). Lungs of chickens infected with *C. psittaci* genotype B show moderate congestion (A) at 6 dpi and diffuse, severe congestion at 21 dpi (B). Infection with *C. psittaci* genotype D results in severe inflammation and lympho-histiocytic and heterophilic infiltration at 6 dpi (C) and in severe congestion, BALT hyperplasia, multifocal bronchitis, and parabronchitis with lympho-histiocytic heterophilic infiltration (D).

and liver compared to infection with strain CP3 ($P < 0.05$). Significantly higher lesion scores due to infection with strain 92/1293 were also observed in kidney tissue during early infection, in trachea, air sacs, pericardium, and liver during midinfection and in spleen and pericardium during late infection (Table 4).

In general, histopathologic lesions in groups 1 and 2 were most severe on days 34 and 10 postinfection, respectively. For group 1, the following was noticed on 34 dpi: 1) Focally, extensive lymphocytic infiltration of the conjunctiva with formation of lymphoid nodules, 2) focally, extensive lymphocytic infiltration of the conchae, 3) focal lymphoid epithelial infiltrate in the trachea, 4) fibrous thickening and multifocal lympho-histiocytic infiltration of the abdominal air sac, 5) severe congestion of the lungs with bronchi-associated lymphoid tissue (BALT) hyperplasia, 6) multifocal lymphocytic infiltration of the liver, and 7) moderate congestion of the spleen.

For group 2, the following was observed on 10 dpi: 1) moderate lympho-histiocytic infiltration of the conjunctiva, 2) mild epithelial hyperplasia of the trachea with heterophilic, lympho-histiocytic infiltration, 3) diffuse, severe, fibrinous, heterophilic, histiocytic inflammation of the thoracic air sac, 4) moderate to severe proliferative, lympho-histiocytic, heterophilic, fibrinous necrotizing inflammation of the abdominal air sac, 5) congestion of the lungs with moderate to severe pneumonia and peribronchitis (histiocytic, heterophilic), 6) pericarditis with diffuse severe heterophilic, lympho-histiocytic infiltration, 7) severe congestion of the liver with multifocal degenerative to necrotizing hepatitis with lympho-histiocytic infiltration, 8) congestion of the spleen with multifocal severe necrotizing fibrinous splenitis, and 9) diffuse lympho-histiocytic infiltration of the ovaries.

DISCUSSION

Avian *C. psittaci* is a risk class 3 pathogen, requiring biosafety level 3 in laboratories willing to isolate the bacterium. Thus, diagnosis of *C. psittaci* in poultry is technically and financially more demanding than diagnosis of other respiratory pathogens. The introduction of nucleic acid amplification techniques made *C. psittaci* diagnosis more feasible for veterinary clinical laboratories. However, *C. psittaci*

Table 4. Histopathologic lesions in early, mid, and late infection stages of *C. psittaci*–infected chickens. Early infection: mean scores of two birds on 2, 4, and 6 dpi; midinfection: mean scores of two birds on 8, 10, 14, and 17 dpi; late infection: mean scores of two birds on 21, 24, 28, and 34 dpi.[A]

	Group 1 (CP3)				Group 2 (92/1293)			
Tissue	Early	Mid	Late	Overall	Early	Mid	Late	Overall
Conjunctiva	0.8	3.0	2.3	2.1	1.2	1.7	1.8	1.6
Conchae	0.7	0.3	0.5	0.5	1.3	1.0	0.3	0.9
Sinus	0.7	0.0	0.0	0.2	1.0	0.0	0.0	0.3
Trachea	0.0	0.0a	0.2	0.1	0.8	2.0b	0.0	0.9
Lung	2.7	2.0	2.8	2.5	2.8	3.0	2.7	2.8
Thoracic air sac	0.7	0.3a	0.0a	0.3a	1.5	2.8b	3.0b	2.4b
Abdominal air sac	0.7	0.5a	1.0	0.7a	1.3	2.7b	3.0	2.3b
Pericardium	0.0	0.3a	0.0a	0.1a	0.7	2.2b	2.0b	1.6b
Spleen	1.2	1.5	1.3a	1.3	1.3	2.2	2.0b	1.8
Liver	0.7	1.0a	0.8	0.8a	0.8	2.5b	2.3	1.9b
Kidney	0.8a	0.7	1.2	0.9	2.0b	1.8	1.3	1.7
Jejunum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ovary/testes	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.2

[A]Histopathologic lesion scores: 1 = mild, 2 = moderate, 3 = severe. Different lowercase letters (a or b) within a row indicate a significant difference between same stages of infection (early, mid, or late) at $P < 0.05$.

diagnosis is for an understandable reason, still not yet routine in veterinary diagnosis. One of the reasons is probably also the fast increasing morbidity and mortality during respiratory disease in poultry. Therefore, antibiotics, often tetracyclines or quinolones, are immediately applied, and diagnosis is often no longer relevant. This might be one of the reasons why *C. psittaci* infections are still neglected and thus underestimated in the poultry industry and why respiratory disease still occurs, notwithstanding intensive (viral) vaccination (infectious bronchitis, Newcastle disease virus, avian metapneumovirus) against respiratory disease, as we know that *C. psittaci* interacts with other respiratory pathogens like, for instance, avian *Escherichia coli* and *Ornithobacterium rhinotracheale* (16,17).

In the past five years, researchers described the occurrence of *C. psittaci* and atypical *Chlamydia* in chickens (8,9,12,24,25,26,27). Thus, *Chlamydia* infections seem to be (re)-emerging in Australian, Chinese, and European broilers and to a lesser extent in layers, although poultry farmers mostly do not seem to be aware of it. Some of these papers document clinical disease in chickens (8,24,25,26,27), while others report no clinical disease in chickens (9,12) but zoonotic transfer and pneumonia in humans (9). Thus, less is known on the pathogenicity of *C. psittaci* strains in chickens. *C. psittaci ompA* genotypes B and D often seem to infect chickens (6,7,19). This is why we conducted this study, examining the pathogenicity of a well-characterized *C. psittaci* genotype B (CP3) and D (92/1293) strain in experimentally infected SPF chickens.

Both strains caused a clinical course of infection, which started with a low incidence and severity of respiratory symptoms during early infection, exacerbated during midinfection to all infected chickens, and diminished again in severity and incidence during late infection. However, respiratory symptoms were more severe in genotype D–infected chickens. Moreover, genotype D also caused anorexia and mortality during midinfection, which was not observed in genotype B–infected chickens. Pharyngeal and cloacal *C. psittaci* excretion was observed in all infected animals, indicative for systemic dissemination as proven by immunofluorescence staining of frozen tissue sections. Cloacal excretion in genotype D–infected chickens was significantly higher during midinfection as compared to early or late infection stages. Strangely, immunohistochemistry could not prove the occurrence of a systemic infection in CP3-infected chickens. Probably, the technique is less sensitive than immunofluorescence staining and/or the LPS of CP3 is no longer detectable after formalin fixation. Nevertheless, histopathologic lesions were present in all infected chickens, and observations on the pathogenicity were in accordance with those observed during other experimental infections in chickens (1,2,14,15).

We could find only 5 other reports on experimental *Chlamydia* infections in chickens. Strains from the following birds and mammals have been used: 1) a budgerigar (Izawa-1; genotype A), 2) a parrot (GCP-1; no genotype specified), 3) a pigeon (P-1041; no genotype specified) (14,15), 4) turkeys (strain C-1; no strain or genotype specified and the Turkey/California/181 strain; no genotype specified) (1,2,4,13), and 5) ruminant strains (B-577, Bo-Yokohama and SPV-789) (14). All these former reports did not use the natural route of infection, namely, inhalation of aerosols. Chlamydiae were directly injected into the air sac or trachea, or chickens were infected orally. The avian strains (10^5 egg lethal $dose_{50}$) used by Takahashi *et al.* (14) induced a generalized infection within 10 dpi followed by death in 8-day-old white leghorn chickens. Strains isolated from psittacine birds were more virulent than the one pigeon strain used, as they caused higher mortality in chickens. Strains derived from ruminants were far less pathogenic to chickens than avian strains.

In the present study, differences in pathology were also observed as genotype D was more virulent than genotype B. Namely, genotype D caused mortality and more severe clinical signs and lesions as compared to genotype B. The same has been observed while examining the developmental cycle of these strains in chicken macrophages (5). Interestingly, similar observations have also been made in SPF turkeys experimentally infected with strain 92/1293 or strain 89/1326, also a pigeon-derived genotype B strain. The incubation period for the genotype B strain was also longer, maximal replication was delayed, the period during which bacteria were observed in the same tissue was also shorter, and tissue tropism also seemed to be less extensive, as compared to an infection with strain 92/1293 (21,22).

At present, we used non-chicken-derived *C. psittaci* genotype B and D strains and demonstrated marked pathogenicity, especially for the genotype D strain in experimentally (aerosol) infected SPF chickens. In the future, experiments on the pathogenicity of chicken derived genotype B and D strains will be conducted.

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